

What is claimed is:

1. A composition comprising a mocarhagin protein at least 95% free of other cobra proteins.
2. The composition of claim 1 wherein said mocarhagin protein is full-length mocarhagin.
3. The composition of claim 1 wherein said mocarhagin protein is a fragment of full-length mocarhagin having mocarhagin proteolytic activity.
4. The composition of claim 1 wherein said mocarhagin protein exhibits an  $IC_{50}$  of less than about 100  $\mu$ g/mL in a neutrophil/HL60 binding inhibition assay.
5. The composition of claim 1 wherein said mocarhagin protein is characterized by at least one characteristic selected from the group consisting of:
  - (a) a molecular weight of approximately 55 kDa under reducing conditions;
  - (b) a molecular weight of approximately 55 kDa under nonreducing conditions;
  - (c) an N-terminal amino acid sequence comprising  
TNTPEQDRYLQAKKYIEFYVVVDNVMYRKY (SEQ ID NO:1);
  - (d) mocarhagin proteolytic activity;
  - (e) the ability to inhibit platelet binding to vWF;

- (f) requirement of calcium ion for activity;
- (g) requirement of zinc ion for activity;
- (h) an activity substantially inhibited by excess EDTA; and
- (i) an activity substantially inhibited by high concentrations of DFP.

6. The composition of claim 1 wherein said mocarhagin protein is capable of cleaving a material selected from the group consisting of anionic polypeptides containing sulfated tyrosine residues, PSGL-1 and GPIb $\alpha$ .

7. A composition comprising a therapeutically effective amount of a composition of claim 1 and a pharmaceutically acceptable carrier.

Sub A3  $\triangleright$  8. A method of treating an inflammatory disease which comprises administering a therapeutically effective amount of a composition of claim 7 to a mammalian subject.

9. A method of inhibiting selectin-mediated binding comprising administering a therapeutically effective amount of a composition of claim 7 to a mammalian subject.

10. A method of isolating mocarhagin from venom, said method comprising:

- (a) subjecting a composition comprising cobra venom to a heparin affinity chromatography column;

- (b) subjecting the eluate from said heparin affinity column to a size exclusion column;
- (c) subjecting the eluate from said size exclusion column to a Mono S column; and
- (d) eluting said mocrhagin from said Mono S column.

11. A composition comprising a protein isolated according to the method of claim 10.

12. The composition of claim 11 further comprising a pharmaceutically acceptable carrier.

Sub A4 13. A method of treating an inflammatory disease which comprises administering a therapeutically effective amount of a composition of claim 12 to a mammalian subject.

14. A method of inhibiting selectin-mediated binding comprising administering a therapeutically effective amount of a composition of claim 12 to a mammalian subject.

15. A composition comprising an antibody which specifically reacts with the mocrhagin of the composition of claim 1 or a fragment thereof having mocrhagin proteolytic activity.

16. The composition of claim 4 wherein said mocarhagin protein exhibits an  $IC_{50}$  of less than about 1  $\mu$ g/mL in a neutrophil/HL60 binding inhibition assay.

17. The composition of claim 1 wherein said mocarhagin protein is homogeneous.

18. The composition of claim 1 wherein the N-terminal sequence of said protein is  
TNTPEQDRYLQAKKYIEFYVVVDNVMYRKYTGKLHVITXXVYEMNALN  
(SEQ ID NO:2).

19. The composition of claim 5 wherein said protein comprises the amino acid sequence of SEQ ID NO:6 from amino acid 192 to amino acid 621.

20. A composition comprising a mocarhagin protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:6;
- (b) the amino acid sequence of SEQ ID NO:6 from amino acid 24 to amino acid 621;
- (c) the amino acid sequence of SEQ ID NO:6 from amino acid 192 to amino acid 621;
- (d) fragments of the amino acid sequence of SEQ ID NO:6 encoding a protein having mocarhagin activity; and

(e) the amino acid sequence encoded by the cDNA insert of clone NMM-1 deposited under accession number ATCC 209588; the protein being substantially free from other mammalian proteins.

21. The composition of claim 20 wherein said protein comprises the amino acid sequence of SEQ ID NO:6.

22. A composition comprising an isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:5;

(b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:5 from nucleotide 78 to nucleotide 1940;

(c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:5 from nucleotide 147 to nucleotide 1940;

(d) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:5 from nucleotide 651 to nucleotide 1940;

(e) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone NMM-1 deposited under accession number ATCC 209588;

(f) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:6;

(g) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:6 from amino acid 24 to amino acid 621;

(h) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:6 from amino acid 192 to amino acid 621;

(i) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:6 encoding a protein having molarhagin activity;

(j) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(h) above;

(k) a polynucleotide which encodes a species homologue of the protein of (f), (g) or (h) above; and

(l) a polynucleotide which hybridizes under stringent conditions to a polynucleotide of (a)-(h) above.

23. A composition of claim 22 wherein said polynucleotide is operably linked to an expression control sequence.

24. A host cell transformed with a composition of claim 23.

25. The host cell of claim 24, wherein said cell is a mammalian cell.

26. A process for producing a protein, which comprises:

(a) growing a culture of the host cell of claim 24 in a suitable culture medium; and

(b) purifying the protein from the culture

27. *sub 15*

A protein produced according to the process of claim 26.

28. The protein of claim 27 comprising a mature protein.

29. A pharmaceutical composition comprising a protein of claim 20 and a pharmaceutically acceptable carrier.

30. A composition comprising a mocarhagin protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:8;

(b) the amino acid sequence of SEQ ID NO:8 from amino acid 24 to amino acid 439;

(c) the amino acid sequence of SEQ ID NO:8 from amino acid 192 to amino acid 439;

(d) fragments of the amino acid sequence of SEQ ID NO:8 encoding a protein having mocarhagin activity; and

(e) the amino acid sequence encoded by the cDNA insert of clone NMM-2 deposited under accession number ATCC 209589; the protein being substantially free from other mammalian proteins.

31. The composition of claim 30 wherein said protein comprises the amino acid sequence of SEQ ID NO:6.

32. A composition comprising an isolated polynucleotide selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:7;
- (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:7 from nucleotide 85 to nucleotide 1401;
- (c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:7 from nucleotide 154 to nucleotide 1401;
- (d) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:7 from nucleotide 658 to nucleotide 1401;
- (e) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone NMM-2 deposited under accession number ATCC 209589;
- (f) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:8;
- (g) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:8 from amino acid 24 to amino acid 439;
- (h) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:8 from amino acid 192 to amino acid 439;
- (i) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:8 encoding a protein having mocrhagin activity;
- (j) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(h) above;



(k) a polynucleotide which encodes a species homologue of the protein of (f), (g) or (h) above; and

(l) a polynucleotide which hybridizes under stringent conditions to a polynucleotide of (a)-(h) above.

33. A composition of claim 32 wherein said polynucleotide is operably linked to an expression control sequence.

34. A host cell transformed with a composition of claim 33.

35. The host cell of claim 34, wherein said cell is a mammalian cell.

36. A process for producing a protein, which comprises:

(a) growing a culture of the host cell of claim 34 in a suitable culture medium; and

(b) purifying the protein from the culture

Sub A6 37. A protein produced according to the process of claim 36.

38. The protein of claim 37 comprising a mature protein.

39. A pharmaceutical composition comprising a protein of claim 30 and a pharmaceutically acceptable carrier.

40. A composition comprising a mocarhagin protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:10;
- (b) the amino acid sequence of SEQ ID NO:10 from amino acid 24 to amino acid 613;
- (c) the amino acid sequence of SEQ ID NO:10 from amino acid 192 to amino acid 613;
- (d) fragments of the amino acid sequence of SEQ ID NO:10 encoding a protein having mocarhagin activity; and
- (e) the amino acid sequence encoded by the cDNA insert of clone NMM-9 deposited under accession number ATCC 209586;

the protein being substantially free from other mammalian proteins.

41. The composition of claim 40 wherein said protein comprises the amino acid sequence of SEQ ID NO:6.

42. A composition comprising an isolated polynucleotide selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:9;
- (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:9 from nucleotide 67 to nucleotide 1905;
- (c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:9 from nucleotide 136 to nucleotide 1905;

(d) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:9 from nucleotide 640 to nucleotide 1905;

(e) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone NMM-9 deposited under accession number ATCC 209586;

(f) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:10;

(g) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:10 from amino acid 24 to amino acid 613;

(h) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:10 from amino acid 192 to amino acid 613;

(i) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:10 encoding a protein having mocarhagin activity;

(j) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(h) above;

(k) a polynucleotide which encodes a species homologue of the protein of (f), (g) or (h) above; and

(l) a polynucleotide which hybridizes under stringent conditions to a polynucleotide of (a)-(h) above.

43. A composition of claim 42 wherein said polynucleotide is operably linked to an expression control sequence

44. A host cell transformed with a composition of claim 43.

45. The host cell of claim 44, wherein said cell is a mammalian cell.

46. A process for producing a protein, which comprises:

- (a) growing a culture of the host cell of claim 44 in a suitable culture medium; and
- (b) purifying the protein from the culture

Sub A7 → 47. A protein produced according to the process of claim 46.

48. The protein of claim 47 comprising a mature protein.

49. A pharmaceutical composition comprising a protein of claim 40 and a pharmaceutically acceptable carrier.

50. A composition comprising a mocrhagin protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:12;
- (b) the amino acid sequence of SEQ ID NO:12 from amino acid 24 to amino acid 521;
- (c) the amino acid sequence of SEQ ID NO:12 from amino acid 192 to amino acid 521;

(d) fragments of the amino acid sequence of SEQ ID NO:12 encoding a protein having mocoarhagin activity; and

(e) the amino acid sequence encoded by the cDNA insert of clone NMM-12 deposited under accession number ATCC 209585; the protein being substantially free from other mammalian proteins.

51. The composition of claim 50 wherein said protein comprises the amino acid sequence of SEQ ID NO:6.

52. A composition comprising an isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:11;

(b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:11 from nucleotide 78 to nucleotide 1640;

(c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:11 from nucleotide 147 to nucleotide 1640;

(d) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:11 from nucleotide 651 to nucleotide 1640;

(e) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone NMM-12 deposited under accession number ATCC 209585;

(f) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:12;

(g) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:12 from amino acid 24 to amino acid 521;

(h) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:12 from amino acid 192 to amino acid 521;

(i) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:12 encoding a protein having mocrhagin activity;

(j) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(h) above;

(k) a polynucleotide which encodes a species homologue of the protein of (f), (g) or (h) above; and

(l) a polynucleotide which hybridizes under stringent conditions to a polynucleotide of (a)-(h) above.

53. A composition of claim 52 wherein said polynucleotide is operably linked to an expression control sequence.

54. A host cell transformed with a composition of claim 53.

55. The host cell of claim 54, wherein said cell is a mammalian cell.

56. A process for producing a protein, which comprises:

(a) growing a culture of the host cell of claim 54 in a suitable culture medium; and

(b) ~~purifying~~ the protein from the culture

Sub A8 57. A protein produced according to the process of claim 56.

58. The protein of claim 57 comprising a mature protein.

59. A pharmaceutical composition comprising a protein of claim 50 and a pharmaceutically acceptable carrier.

60. A composition comprising a mocarhagin protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:14;

(b) the amino acid sequence of SEQ ID NO:14 from amino acid 24 to amino acid 592;

(c) the amino acid sequence of SEQ ID NO:14 from amino acid 192 to amino acid 592;

(d) fragments of the amino acid sequence of SEQ ID NO:12 encoding a protein having mocarhagin activity; and

(e) the amino acid sequence encoded by the cDNA insert of clone NMM-13 deposited under accession number ATCC 209584; the protein being substantially free from other mammalian proteins.

61. The composition of claim 60 wherein said protein comprises the amino acid sequence of SEQ ID NO:6.

62. A composition comprising an isolated polynucleotide selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:13;
- (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:13 from nucleotide 83 to nucleotide 1858;
- (c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:13 from nucleotide 152 to nucleotide 1858;
- (d) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:13 from nucleotide 656 to nucleotide 1858;
- (e) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone NMM-13 deposited under accession number ATCC 209584;
- (f) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:14;
- (g) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:14 from amino acid 24 to amino acid 592;
- (h) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:14 from amino acid 192 to amino acid 592;
- (i) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:14 encoding a protein having mocrhagin activity;
- (j) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(h) above;



(k) a polynucleotide which encodes a species homologue of the protein of (f), (g) or (h) above; and

(l) a polynucleotide which hybridizes under stringent conditions to a polynucleotide of (a)-(h) above.

63. A composition of claim 62 wherein said polynucleotide is operably linked to an expression control sequence.

64. A host cell transformed with a composition of claim 63.

65. The host cell of claim 64, wherein said cell is a mammalian cell.

66. A process for producing a protein, which comprises:

(a) growing a culture of the host cell of claim 64 in a suitable culture medium; and

(b) purifying the protein from the culture

67. A protein produced according to the process of claim 66.

68. The protein of claim 67 comprising a mature protein.

69. A pharmaceutical composition comprising a protein of claim 60 and a pharmaceutically acceptable carrier.

70. A composition comprising a mocarhagin protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:16;
  - (b) the amino acid sequence of SEQ ID NO:16 from amino acid 62 to amino acid 462;
  - (c) fragments of the amino acid sequence of SEQ ID NO:16 encoding a protein having mocarhagin activity; and
  - (d) the amino acid sequence encoded by the cDNA insert of clone NMM-3 deposited under accession number ATCC 209587;
- the protein being substantially free from other mammalian proteins.

71. The composition of claim 70 wherein said protein comprises the amino acid sequence of SEQ ID NO:6.

72. A composition comprising an isolated polynucleotide selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:15;
- (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:15 from nucleotide 3 to nucleotide 1388;
- (c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:15 from nucleotide 186 to nucleotide 1388;

(d) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone NMM-3 deposited under accession number ATCC 209587;

(e) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:16;

(f) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:16 from amino acid 62 to amino acid 462;

(g) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:16 encoding a protein having molarhagin activity;

(h) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(g) above;

(i) a polynucleotide which encodes a species homologue of the protein of (e) or (f) above; and

(j) a polynucleotide which hybridizes under stringent conditions to a polynucleotide of (a)-(g) above.

73. A composition of claim 72 wherein said polynucleotide is operably linked to an expression control sequence.

74. A host cell transformed with a composition of claim 73.

75. The host cell of claim 74, wherein said cell is a mammalian cell.

76. A process for producing a protein, which comprises:
- (a) growing a culture of the host cell of claim 74 in a suitable culture medium; and
  - (b) purifying the protein from the culture

Sub A10 77. A protein produced according to the process of claim 76.

78. The protein of claim 77 comprising a mature protein.

79. A pharmaceutical composition comprising a protein of claim 70 and a pharmaceutically acceptable carrier.

80. A composition comprising a mocrhagin protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:18;
  - (b) the amino acid sequence of SEQ ID NO:18 from amino acid 197 to amino acid 621;
  - (c) fragments of the amino acid sequence of SEQ ID NO:18 encoding a protein having mocrhagin activity; and
  - (d) the amino acid sequence encoded by the cDNA insert of clone NMM-9ek deposited under accession number ATCC 209583;
- the protein being substantially free from other mammalian proteins.

81. The composition of claim 80 wherein said protein comprises the amino acid sequence of SEQ ID NO:6.

82. A composition comprising an isolated polynucleotide selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:17;
- (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:17 from nucleotide 67 to nucleotide 1929;
- (c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:17 from nucleotide 655 to nucleotide 1929;
- (d) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone NMM-9ek deposited under accession number ATCC 209583;
- (e) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:18;
- (f) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:18 from amino acid 197 to amino acid 621;
- (g) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:18 encoding a protein having mocrhagin activity;
- (h) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(g) above;

(i) a polynucleotide which encodes a species homologue of the protein of (e) or (f) above; and

(j) a polynucleotide which hybridizes under stringent conditions to a polynucleotide of (a)-(g) above.

83. A composition of claim 82 wherein said polynucleotide is operably linked to an expression control sequence.

84. A host cell transformed with a composition of claim 83.

85. The host cell of claim 84, wherein said cell is a mammalian cell.

86. A process for producing a protein, which comprises:

(a) growing a culture of the host cell of claim 84 in a suitable culture medium; and

(b) purifying the protein from the culture

Sub All 87. A protein produced according to the process of claim 86.

88. The protein of claim 87 comprising a mature protein.

89. A pharmaceutical composition comprising a protein of claim 80 and a pharmaceutically acceptable carrier.

90. A composition of claim 22 wherein said polynucleotide encodes a protein containing an enterokinase cleavage site.

91. A composition of claim 32 wherein said polynucleotide encodes a protein containing an enterokinase cleavage site.

92. A composition of claim 42 wherein said polynucleotide encodes a protein containing an enterokinase cleavage site.

93. A composition of claim 52 wherein said polynucleotide encodes a protein containing an enterokinase cleavage site.

94. A composition of claim 62 wherein said polynucleotide encodes a protein containing an enterokinase cleavage site.

95. A composition of claim 72 wherein said polynucleotide encodes a protein containing an enterokinase cleavage site.

Ad Ad